EXHIBIT 6

1: Int J Dev Neurosci 1996 Nov;14(7-8):823-39

Characterization of olfactory receptor neurons and other cell types in dissociated rat olfactory cell cultures.

Pixley SK.

Dept of Cell Biology, Neurobiology and Anatomy, University of Cincinnati College of Medicine, Ohio, USA. sarah.pixley@uc.edu

In dissociated cell cultures, control over the cellular environment facilitates study of the differentiation of mature cellular phenotypes. Central to this approach is a rigorous characterization of the cells that reside in culture. Therefore, we have used a battery of cell type-specific antibody markers to identify the cell types present in dissociated cultures of olfactory mucosal cells (containing cells from both the epithelium and lamina propria). To identify olfactory receptor neurons in the cultures, staining with antibodies against neuron-specific tubulin was compared to staining with antibodies to neuron-specific enolase, the neural cell adhesion molecule, N-CAM, and the adhesion molecule, LI. Staining of mature olfactory neurons in culture, with an antibody against the olfactory marker protein, was compared to staining with antibodies to carnosine. In contrast to tissue section staining, the overlap between carnosine and olfactory marker protein staining was not complete. Olfactory nerve glial cells were immunoreactive for the S100 beta protein and nestin, an intermediate filament found in early neuronal progenitor cells and Schwann cells. Antibodies to nestin did not label olfactory neurons or progenitor cells. An antibody to an oligodendrocyte-Schwann cell enzyme, 2',3'-cyclic nucleotide 3'-phosphodiesterase, did not label olfactory glia, but did label oligodendrocyte-like cells that appeared to be derived from the CNS glial feeder layer. An antibody against the heavy (200 kDa) neurofilament protein stained a minor subset of cells. The cultures also contained muscle cells, cartilage cells and macrophages (and/or microglia). These results demonstrate that multiple cell types either maintain or re-establish differentiated, cell type-specific phenotypes in dissociated olfactory cell cultures.

PMID: 9010728 [PubMed - indexed for MEDLINE]